PATENT
4518-101P

IN THE U.S. PATENT AND TRADEMARK OFFICE

APPLICANT:	ECKERT, Helmut et al.	CONF:	8183
SERIAL NO.:	10/090,663	GROUP:	1642
FILED:	March 5, 2002	EXAMINER:	HARRIS, A.
FOR:	MONOCLONAL ANTIBODIES AND THEIR USE		

DECLARATION SUBMITTED UNDER 37 C.F.R. § 1.132

Honorable Commissioner
Of Patents and Trademarks
Washington, D.C. 20231

December 11, 2002

Sir:

I, Dr. Hans Loibner of IGENEON Krebs- Immuntherapie Forschungs- und Entwicklungs- AG Vienna, Austria, do hereby declare the following:

I have attached a copy of my curriculum vitae to this Declaration.

I am a founder of IGENEON Krebs- Immuntherapie Forschungs- und Entwicklungs- AG.

I am familiar with the above referenced patent application, as well as the development, usages and properties of anti-idiotypic antibodies.

The following comments are offered in support of the patentability of the instant invention.

The invention of the application is directed to the generation, production and characterization of murine monoclonal internal image anti-idiotypic antibodies (Ab2) to monoclonal antibodies BR55-2 (Ab1). In addition, the invention uses these anti-idiotypic antibodies for active therapeutic and prophylactic immunization against cancer of epithelial origin and small cell lung cancer, as well as against diseases caused by HIV-infection.

An anti-id antibody is generally defined as having the ability to mimic the nominal antigen and to provoke an immune response directed against the nominal antigen after vaccination with the anti-id antibody. This comes about in the following manner.

First, an antigen, such as the Lewis Y carbohydrate, has a unique exterior pattern of receptacles and connectors. An antibody (Ab1) produced against the antigen can be thought of as the mirror image of the antigen. That is, if the antigen has a receptacle, the antibody has a connector and vice versa. The antibody has an idiotype, which is a set of one or more pieces specific to an antibody.

Next, if the antibody itself is used as an antigen, an anti-idiotypic antibody (anti-id Ab or Ab2) is produced. Again, the anti-id Ab can be thought of as the mirror image of the antigen, in this case the original antibody. But this means that the anti-id Ab is substantially identical to the original antigen (the Lewis Y carbohydrate). Consequently, when the anti-id Ab is used as an antigen, an antibody produced against it (i.e. Ab3) can also attack the original antigen (the Lewis Y carbohydrate). Thus, the anti-id Ab acts as an active immunization agent, stimulating the immune system to produce antibodies that attack, rather than as a passive immunization, such as injecting antibodies already formed against the targeted antigen.

The anti-id BR55-2 MAbs of the current invention (see page 9, paragraph 3) meet this description. That is, although the anti-id Abs are protein entities, they have the structural characteristics of the Lewis Y carbohydrate and, when acting as antigens, stimulate the production of antibodies that can attack either the anti-id Ab or the Lewis Y antigen (LeY). Thus, they can be used as a surrogate tumor antigen and have the ability to generate an Ab3 response to the nominal tumor associated antigen, in this case LeY.

In the past, anti-id Ab (Ab2) procedures produced a mixture of Ab2 antibodies at best. For example, Steplewski (USP 4,971,792) suggests isolating hybridomas secreting monoclonal antibodies with the specificity of MAb BR55-2 by using anti-idiotypic screening as detailed by Potocnjak et al. (Science, 1982, 215: 1637-1639; copy enclosed). Here Potocnjak et al. do not describe the criteria of the screen needed. Without these criteria, one cannot guarantee obtaining an antibody suitable for medical purposes. Neither would one know how to select antibodies for clinical studies with the appropriate binding characteristics, which are recited within the instant claims.

The deficiency in the Potocnjak et al. reference is similar to that of the Loibner reference (7th International Congress of Immunology, 1989, Abstract), which only teaches producing a mixture of antibodies. Furthermore, the combination of these two references does not cure the inadequacies of the individual references.

In contrast, the present invention makes It possible to reproducibly obtain the anti-idiotypic antibody BR55-2#E4, which has an inhibition capacity of more than 95% for BR55-2 at a concentration of less than or equal to a ten (10) fold excess of Ab2 to Ab1. This was achieved using the procedure set forth in the application on page 7 and 8. Here, the $F(ab')_2$ –fragment of the BR55-2, murine IgG3 antibody was coupled to Keyhole Limpet Hemocyanin (KLH). This conjugate was used to immunize mice and hybridoma cells isolated. The supernatants of the cultured hybridoma cells was screened for (a) high rate of secretion of murine IgG, (b) binding to a switch variant of the $F(ab')_2$-fragment of BR55-2/murine IgG3 in a mixture containing an excess of unspecific mouse-IgG and (c) inhibition of binding of BR55-2/murine IgG2a to Lewis Y antigen positive SKBR5 cancer cells. Only hybridomas that produce IgG with an inhibition capacity of more than 95% were chosen. As a result, several anti-idiotypic antibodies with very similar specificities were obtained and purified by immunoaffinity chromatography.

The experiment described in attached Appendix C identifies six anti-id Abs that demonstrate these internal image properties. These six anti-id Ab are from a group of 18 initially selected clones from a single immunization fusion experiment. The results of the experiment are shown in the graph, which demonstrates that each of the anti-idiotypic antibodies (E4, C11, B3, B9, G6 and G9, which are also described in the application), when used as vaccine in Rhesus monkeys, induced antibodies against LeY positive SKBR5 tumor cells. Figure 3 of the application shows representative binding results that indicate essentially complete inhibition of binding of the BR55-2 antibody to SKBR5 cells that present LeY. Thus, the present invention represents a significant advancement over the prior art.

The undersigned hereby declares that all statements made herein based upon knowledge are true, and that all statements made based upon information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

DATED: 11 12 2002

Dr. Hans Loibner

Enclosures: As stated above

Curriculum Vitae
Hans Loibner, Ph.D.

Date of Birth: October 16, 1947
Place of Birth: Vienna, Austria
Citizenship: Austria

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Family status: Divorced, 4 children

Education: Primary school
High school
University Vienna: Chemistry

1975 - 1977 Thesis at Department of Organic Chemistry,
Prof.Dr.E.Zbiral, University Vienna, in synthetic organic chemistry

1977 Ph.D.

1977 - 1979 **Postdoctoral fellow** at Sandoz Forschungsinstitut (SFI):
Chemical synthesis of new aminoglycoside antibiotics with improved properties (active against resistant strains and less toxicity);
Creation of an efficient system for preparative medium pressure high performance liquid chromatography.

1979 - 1984 **Head of a chemistry laboratory** at SFI:
Synthesis of aminoglycoside antibiotics as above;
synthesis of immunostimulants.

1981-1984 **Head of working group "Aminoglycoside-Antibiotics"**
Coordination of all activities in chemistry, biology and toxicology for characterization of novel aminoglycoside antibiotics and identification of development candidates.

1984 - 1986 **Head of Department "Molecular Enzymology"** at SFI:
Main tasks:

- Mechanism based strategies for discovery and characterization of substances for immunostimulation
- Enzymatic preparative synthesis and biological characterization of lipid-A analogs
- Enzymological and cellular tests for assessment of macrophage stimulation
- Protein chemistry, protein purification and -analytics

1986 - 1989 **Head of Department "Antibodies"** at SFI:
Main tasks:

- Discovery, profiling and development of antibody-based immunotherapies against cancer, in particular tumor specific monoclonal antibodies for passive immunotherapy and anti-idiotypic antibodies for therapeutic vaccination
- Generation and characterization of new monoclonal antibodies for all research purposes of the institute
- Fermentation of bacteria as well as of mammalian cells for production of proteins
- Protein purification and -analytics; automated peptide synthesis.

1987 - 1994 **Head of international project teams**
Responsibility for coordinated international preclinical and in particular clinical development of projects in specific cancer immunotherapy
(ABL 364: monoclonal antibody for passive immunotherapy, SCV 106: anti-idiotypic antibody vaccine for therapeutic vaccination), together with internal departments and involved external clinical centers.

1990 - 1994 **Head of Department "Special Projects"** at SFI:
Main tasks:
- Assessment of immunological and serological parameters of cancer patients in the context of clinical studies with cancer immunotherapy approaches developed at SFI
- Generation and preclinical profiling of new projects in specific cancer immunotherapy

1995 **Head of Department Genetics** at SFI
Main tasks:
- Establishment of highly automatized methods for comprehensive analysis of expressed genes as novel tool for identification of molecular targets for disease intervention in all disease areas of interest for Sandoz Pharma
- Shaping of the collaboration with Prof.Lehrach, MPI Berlin regarding Fingerprinting analysis of expressed genes
- Responsibility for preclinical development of an anti-idiotypic antibody vaccine against epithelial cancer (MMA 383) generated at SFI in the context of an international project team

1996 **Head of MMA 383 Support Group** at SFI
Main task:
Support of further development of the anti-idiotypic antibody vaccine MMA 383, aiming at initiation of clinical proof of concept trials 1/1997

1997 - 1999 **Head of Oncology Group Vienna** at Novartis Forschungsinstitut
Main tasks:
Support of the anti-idiotypic antibody vaccine MMA 383 as above. Basic research in the area of active specific immunotherapy.

1999 - 2001 **Head of Igeneon GmbH**
Development of cancer vaccines and immunotherapies

2001 - **CEO, Igeneon Krebs-Immuntherapie Forschungs-und Entwicklungs-AG**

Appendix C:

One Rhesus monkey each was vaccinated s.c. on days 1, 8, 15 and 29 with each 0.1 mg/kg purified anti-id antibodies produced by hybridomas C11, B3, B9, G6 and G9, adsorbed each on 1.67 mg aluminum hydroxide. Three Rhesus monkeys were vaccinated in the same way (same dose, formulation and schedule) with the purified anti-id antibody produced by hybridoma E4. Sera were taken on days 1, 15, 29 and 43 and analyzed for the presence of antibodies binding to Lewis Y positive SKBR5 breast cancer cells, as described below. The results of this experiment are shown in the attached Figure.

Microtiter plates (Nunc F96 Maxisorb; 442404) are incubated overnight at 4°C with 100 µL/well of a suspension of the SKBR5 cells in medium A (2 x 10^6 cells/mL). Usually the cells sufficiently stick onto the plate for further handling (if not, before removing a supernatant it is always necessary to centrifuge the plates for 5 minutes at approx. 210 g). After removal of the supernatant (careful suck off), the cells are fixed with 50 µL glutardialdehyde/well (0.1% in physiological saline) for 5 minutes at room temperature, the supernatants are removed (careful suck off), 200 µL/well of PBS def./1% BSA/0.1% NaN_3 added and stored at 4°C up to 4 weeks. Supernatants are removed (careful suck off) and the plates are washed twice with 200 µL/well of PBS def. containing 0.05% Tween 20. 100 µL aliquots of sera in appropriate predilutions with PBS def. are added to the cells and the plates are incubated for 1 hour at 37°C. Unbound Ig is washed out twice with 100 µL/well of ice-cold PBS def. containing 0.05% Tween 20 (careful suck off) and 100 µL aliquots of goat-anti-human Ig/peroxidase, 1:1000 in PBS/2% FCS are added (Zymed; 62 - 8320). After incubation for 45 minutes at 37°C the wells are washed three times with the PBS def./Tween 20 solution mentioned above (careful suck off) and then 100 µL of the substrate solution is added to each well. After approx. 8 minutes, color development is stopped by addition of 50 µL of 4N H_2SO_4/well. Binding of Ig to the cells is determined by measuring extinction at 492 nm (reference measurement 620 nm). Results are plotted in a graph with OD at the y-axis and dilution factor at the x-axis. For calculation of arbitrary titer units, the area under the titration curves is determined.

Medium A:	RPMI 1640 + 2 g/l $NaHCO_3$
	100 U/mL penicillin G
	100 µg/mL streptomycin sulfate
	4 mM glutamine
	5% FCS (heat-inactivated)
PBS deficient:	138.0 mM NaCl
	1.5 mM KH_2PO_4
	2.7 mM KCl
	6.5 mM Na_2HPO_4
	pH 7.2
Substrate solution:	40 mg o-phenylenediamine dihydrochloride
	100 mL staining buffer
	20 µL H_2O_2 30%

signed by Hans Loibner
on ..., March 15, 2000

signed by Evelyne Janzek
on ..., March 15, 2000

Induction of serum Ig against LeY positive SKBR5 tumor cells by vaccination of Rhesus monkeys with different anti-id antibodies against BR55-2 (cell-ELISA)